Soft-bottom intertidal ecosystems shaped by ecosystem engineers

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Chapter 7

7

Hierarchical habitat modification shapes food web structure over long timescales

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Abstract

Food webs are often studied in ignorance of non-trophic interactions like mutualism and habitat modification, and their structure is therefore considered as a function of the properties of the trophic network itself. Using a meta-analysis combined with in-depth investigations of a seagrass ecosystem, we reveal that hierarchical habitat modification by seagrasses and burrowing crabs transform simple intertidal food webs into a complex mosaic of linked inter- and subtidal food webs over long timescales. We found that colonizing seagrass patches, that accumulate silt and form 3D structure, and burrowing crabs, that create large intertidal pools in the accumulated silt layer, dramatically alter food web structure and its temporal development. Our empirical findings show that non-trophic interactions are strongly interwoven with trophic networks, changing network dynamics over time. These outcomes provide grounds for new approaches to ecological network analyses and ecosystem conservation.

In preparation for publication

One of the great challenges in ecology is to elucidate how species interact with each other and their environments, and how these interactions drive the assembly of complex ecological networks. Ever since Darwin (1859) coined the term 'web of life', trophic interactions, the resulting food web structure and its stability have been intensively studied (e. g. May 1973; Pimm 1982; Cohen *et al.* 1990; Neutel *et al.* 2007). However, as Darwin (1859) already noted, trophic relations are only one of many types of interactions through which species can affect each other. Non-trophic interactions, like mutualism and habitat modification can also strongly affect food webs by influencing diversity, community structure or even by facilitating the persistence of entire communities (e.g. Jones *et al.* 1994; Bruno and Bertness 2001; Stachowicz 2001). However, the multi-trophic structure of natural food webs is often studied in ignorance of such non-trophic interactions (as emphasized by Berlow 2004; Ings *et al.* 2009; Olff *et al.* 2009; Fontaine *et al.* 2011) and although integration of trophic and non-trophic interactions into a single framework has recently been gaining attention, the studies addressing this issue have remained of a theoretical nature (Arditi *et al.* 2005; Goudard and Loreau 2008; Kefi *et al.* 2012). Additionally, it is increasingly recognized that understanding the assembly of complex ecological networks requires the inclusion of temporal dynamics (Blonder *et al.* 2012), since food web structure is often not static, but changes over time due to for instance succession (Schoenly and Cohen 1991; Neutel *et al.* 2007; Schrama *et al.* 2012). Besides succession, facilitation through habitat modification also has the potential to affect food web structure over time (Hastings *et al.* 2007), especially when multiple habitat modifying species act hierarchical over time in the form of a facilitation cascade (Altieri *et al.* 2007; Thomsen *et al.* 2010; Angelini and Silliman 2014). Despite the potential importance of both habitat modification and temporal dynamics in structuring food webs, empirical studies that integrated food web assembly with habitat modification in a temporally explicit manner are lacking to our knowledge. Therefore, even after 150 years the question remains: how (much) do non-trophic interactions affect the development and structure of real food webs over time?

Here, we addressed this question by investigating how non-trophic facilitation by habitat modifying species affects food web structure and biodiversity over a long time scale. As a first indication of how habitat modifiers impact food web structure, we compared the number of species across multiple trophic levels in food webs of unmodified and modified habitats in a 18-study meta-analysis spanning 16 types of marine, freshwater and terrestrial systems (Supp. Mat., table S1). Results revealed consistently higher species numbers in food webs when those feeding networks occurred in habitats generated by habitat modifiers. On average, species richness was over 3.5 times higher in modified habitats compared to unmodified areas (table S1).

Next, we investigated the mechanisms driving food web structure in the seagrass meadows on the intertidal flats of Banc d'Arguin (Mauritania, West-Africa). Seagrasses are important ecological components of coastal zones worldwide that

serve as a keystone habitat for thousands of species including large numbers of bivalves, gastropods, shrimp, fish and waterbirds (Beck *et al.* 2001; Larkum *et al.* 2006) and they are habitat modifiers in the sense that they modify their surroundings by providing aboveground structure through their shoots and by trapping suspended sediments and organic particles (Bos *et al.* 2007; van der Heide *et al.* 2007). Here, we present a series of food webs, sampled over a natural gradient of seagrass colonization in the Banc d'Arguin. We identified three distinct stages of colonization, providing us with a unique opportunity to investigate the interplay of habitat modification and food web assembly dynamics over time. Using satellite imagery (U.S. Geological Survey; 1973–2010) combined with ground truthing, we classified (1) bare, (2) colonizing (~3 year-old) seagrass and (3) long-term (>37 year-old) stable seagrass habitats (fig. 7.1. A t/m C, table S2, Supp. Mat.). Next, we selected four replicate sites for each habitat type, with a random spatial distribution and similar environmental characteristics (fig. S1 & table S3).

To examine the effects of seagrasses on their associated community and the abiotic environment (i.e. habitat modification) we determined seagrass properties and sediment conditions in the field (table S4). All four replicates of bare habitat were characterized by coarse, sandy substrate. The sites with 3-year-old, colonizing seagrasses consisted of a mosaic of seagrass patches (*Zostera noltii*) alternating with bare sediment. Average seagrass patch cover at these sites was 46.3 ± 10.3 % (mean ± SE) with a shoot density of 6246 ± 510 shoots per square meter within these patches. Similar to bare habitat, sediments in uncolonized areas consisted of coarse sands. Seagrass patches, however, had accreted a 0.05 ± 0.01 m silt layer during 3 years (fig. 7.1.D). Shoot density in the long-term stable meadows (7167 ± 575 shoots m^{-2}) did not differ much from those measured in patches at the colonizing sites, but overall seagrass cover increased significantly to 87.5 ± 3.2% ($F_{2,9} = 49.2$, $P < 0.001$). The silt layer in the long-term stable meadows had gained a height of 0.9 ± 0.2 m (fig. 7.1D), even though the mean elevation in these habitats did not differ from bare and colonizing sites (table S3).

Apart from a high seagrass cover and a thick silt layer, long-term stable meadows were also typified by a high number of intertidal pools in the silt layer (82.8 ± 9.2 pools ha^{-1}, 0.19 ± 0.01 m depth) that stand for 30 ± 4% subtidal coverage within the intertidal habitat. Using satellite image analysis, we found that these pools were spatially stagnant, but on average increased slowly in size over time (mean $size_{2004} = 37.6 \pm 2.4\ m^2$, mean $size_{2011} = 47.0 \pm 2.7\ m^2$, $t = -3.307$, $n = 60$, $P < 0.01$, Supp. Mat.). Because experimental depressions in this area have been shown to quickly disappear (van der Laan and Wolff 2006), these outcomes suggest that the pools represent the outcome of an interaction between sediment accretion by seagrasses and bioturbation by marine animals. Subsequent field surveys revealed high abundances of burrowing crabs (*Callinectes marginatus*) in the long-term stable meadows, as indicated by the number of holes (3300 ± 427 ha^{-1}, fig.

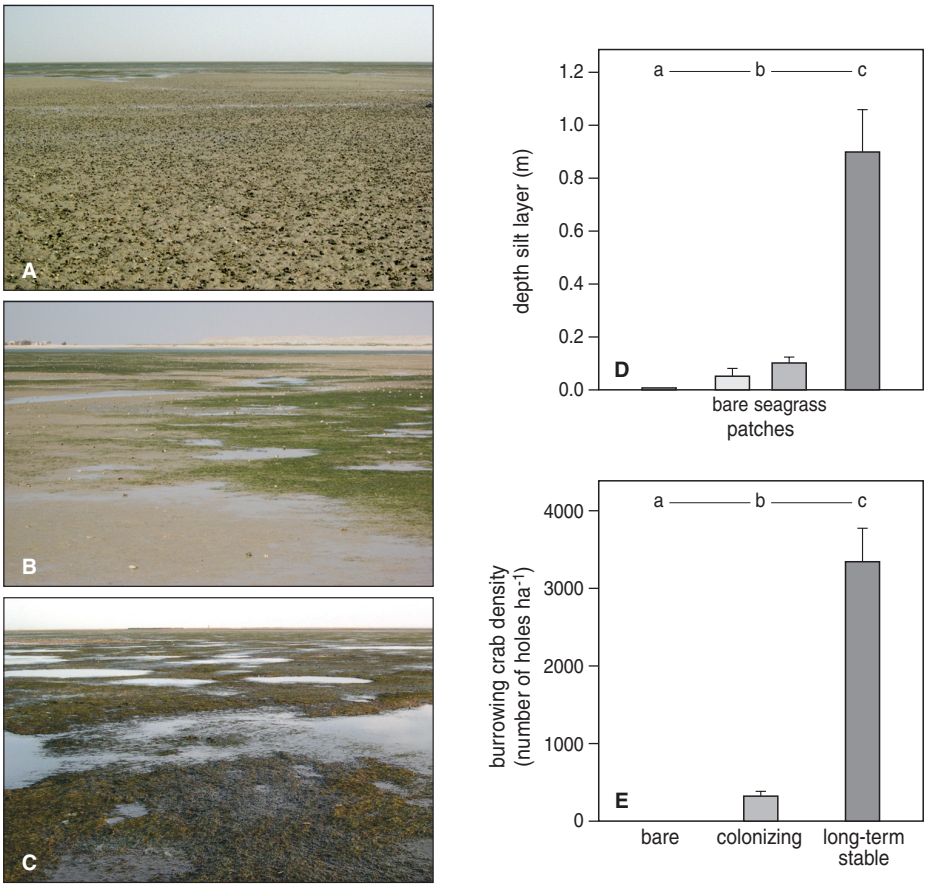

Figure 7.1 Study system. Habitat type: Bare (**A**), Colonizing (**B**) and Long-term stable (>37 years)(**C**). (**D**) Silt accumulation in each habitat. Within colonizing seagrass meadows, the silt layer was measured in seagrass patches and in bare patches, but for statistical analysis bare patches and seagrass patches were averaged. Silt accumulation differed significantly between habitats (One-way ANOVA; $F_{2,9} = 98.3$, $P < 0.001$). (**E**) Burrowing crab densities in each habitat with differing densities for each habitat (Kruskal-Wallis; $\chi^2 = 9.3$, $P = 0.009$).

7.1E), and low densities of other potentially bioturbating animal species, such as fish and birds (supplementary text, tables S5 & S6). Moreover, we found a strong positive correlation between crab hole numbers within the pools and pool size ($R^2 = 0.74$, $t = 17.1$, $P < 0.001$; fig. S2). Finally, an onsite cage experiment (fig. S3) demonstrated that within 24 hours these crabs were able to dig a hole (0.05 ± 0.02 m^2; $n = 3$) in the silt layer of an undisturbed seagrass meadow. This illustrates that crabs can create pools rather than only colonize them. Overall, these results combined indicate that bioturbation by crabs in the thick silt layer, possibly exacerbated by feeding birds and fish, is the driving mechanism behind the formation of these pools.

We investigated the consequences of this hierarchical organization of habitat modification by seagrasses and burrowing crabs on the food web structure by identifying and collecting all resident (i.e. not migrating with the tides) species at all sites during low tide. Nitrogen and carbon stable isotope values were determined for each species at each site and we constructed trophic interaction matrices based on literature, abundance and isotope data, and mixing models (Supp. Mat. & table S7). Next, we calculated species richness, the number of links per species and connectance (the realized fraction of all possible links) for each site as indicators for food web structure (May 1972; Williams and Martinez 2000; Neutel *et al.* 2007; Gross *et al.* 2009). We found that all three indicators strongly responded to the introduction of habitat modifiers (fig. 7.2 & table S8). Both species richness (S) and the number of links per species (L/S) strongly increased. Species richness and the number of links per species (L/S) increased 1.5 times from bare to colonizing seagrass and was another 2 and 1.5 times enhanced in long-term stable seagrass meadows, respectively (table S8). Connectance (C; defined as L/S2) on the other hand, displayed the opposite trend, decreasing by 0.6 times as the systems matured from bare to colonizing seagrass and long-term stable seagrass meadows, indicating that habitat modification by seagrasses and burrowing crabs strongly affect food web properties over time (fig. 7.2 & table S8).

To determine how much habitat modifiers impacted food web structure through non-trophic and trophic pathways, we examined the abiotic and dietary requirements of each species (Supp. Mat.). First, we investigated the importance of modification by crabs in long-term stable meadows by removing species from our dataset that depend on the intertidal pools and by comparing the newly calculated values of species richness, number of links per species and connectance to those in colonizing seagrasses and bare habitat. We found that species richness, number of links per species no longer differed between long-term stable and colonizing seagrass habitats (fig. 7.2 & table S8). However, compared to bare habitat, these two indicators were still around 1.5 times enhanced. Connectance no longer differed between all three habitats (fig. 7.2 & table S8). Second, as a test of their trophic effect, we instead removed crabs themselves and their trophic links from the matrix, and as a result also species that were only trophically connected to crabs. Contrastingly, whereas removal of non-trophic effects changed food web indicators by 44% on average, the change in indicators resulting from the removal of the trophic effect of crabs was nearly an order of magnitude lower; 4% on average.

Next, we tested the importance of seagrass modification by also removing species from our dataset that depended on either silt accumulation or aboveground structure provided by the seagrasses. Results show that all three food web indicators in both stable and colonizing habitats converged towards bare, unmodified habitats. In this case, species richness, the number of links per species and connectance no longer differed between any of the habitat types (fig. 7.2 A t/m C &

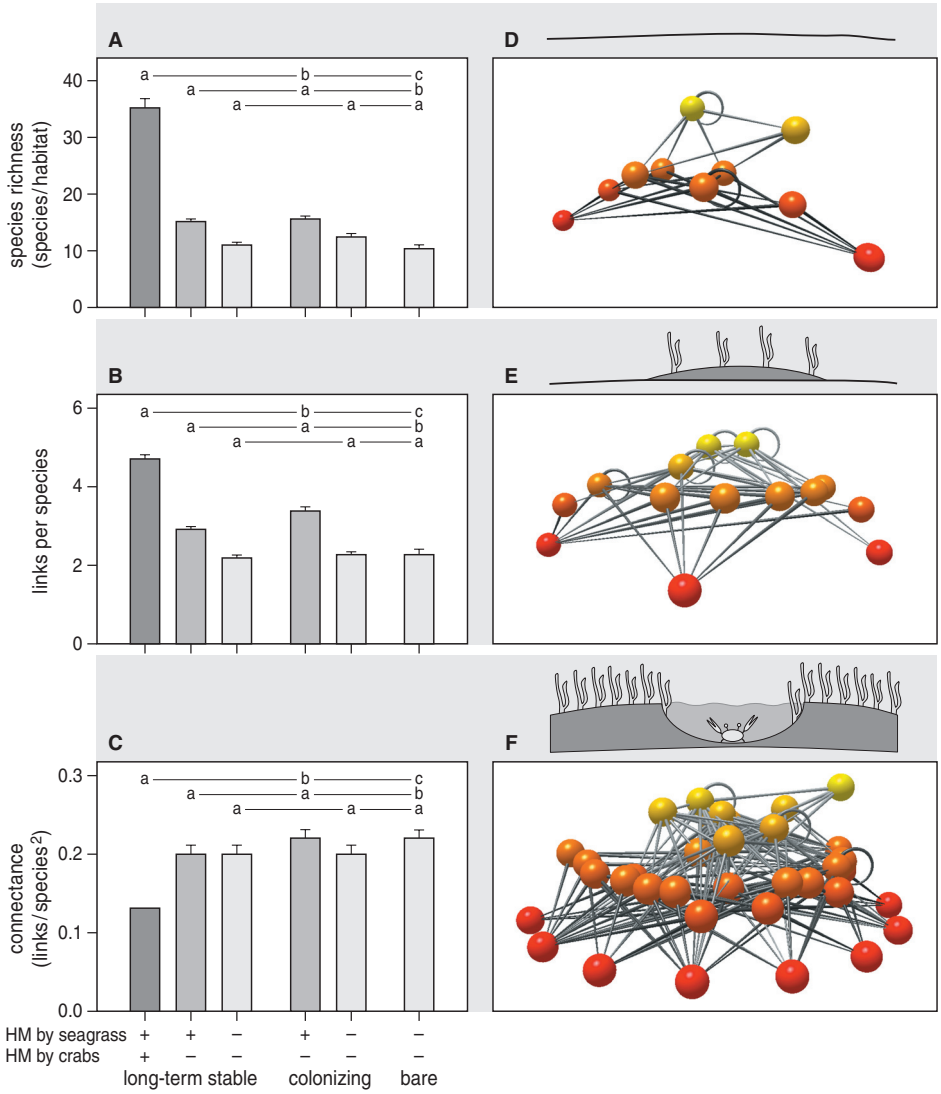

Figure 7.2 (**A**) Species richness, (**B**) links per species and (**C**) connectance for the different habitat types and habitat modification effects (HM). Letters indicate posthoc grouping; Error bars represent SEM. S and L/S are significantly higher in modified habitat, whereas C is lower in long-term stable habitats (see tables S8). Stepwise removal of species from the interaction matrix that are dependent on habitat modification (HM by crabs and HM by seagrass) increased similarity to unmodified, bare habitat. Figures (**D**), (**E**) and (**F**) show a graphical representation of the food webs in bare, colonizing and long-term stable habitat, respectively. Bare sites are typified by a relatively simple food web (**D**). Food webs have higher species richness and link density in habitat colonized by primary habitat modifiers (seagrass, **E**) and these effects are further enhance by secondary habitat modifiers (crabs, **F**) in long-term stable habitat. Node color gradually changes from red (primary producer) to yellow with increasing trophic level. Food web images were made with Network3D (Yoon *et al*. 2004; Williams 2010).

table S8), suggesting that in the absence of habitat modification by seagrass, diversity and complexity do not increase even when trophic relations with seagrass are left intact. Apart from connectance, which appears largely unaffected by both non-trophic and trophic interactions by seagrass, removal of non-trophic effects of seagrass changed food web indicators by 24% on average, whereas removal of trophic effects of primary habitat modifiers changed food web indicators by 4% on average.

Our empirical findings clearly demonstrate that non-trophic facilitation by habitat modification can strongly affect food web structure and its temporal development, not only by enhancing species numbers, but also by changing the linkages and connectance among species. Moreover, we found these non-trophic effects by habitat modifiers to be more important for food web structure than their trophic effects. Still, even though we were able to determine the effect of habitat modification on trophic dynamics, we most likely underestimated the importance of other non-trophic relations since other non-trophic interactions may further complicate the ecological network in our system (van der Heide *et al.* 2012).

Overall, our empirical findings emphasize that food web structure can be strongly controlled by non-trophic interactions and that such interactions can be highly complex in terms of being hierarchical and temporal. Furthermore, since May (1972) mathematically demonstrated that randomly assembled food webs become less robust as their complexity increases, food web structure and stability have been typically analyzed as a function of the properties of the network itself, like the number of species and links, connectance (Williams and Martinez 2000; Dunne *et al.* 2002; Gross *et al.* 2009; Dunne *et al.* 2013) or the strength of trophic loops in the network (Neutel *et al.* 2002). Our findings indicate that food web structure and temporal dynamics can to a large extent be determined by interactions outside the trophic network, yet intrinsic to the species of the system. We therefore conclude that integration of non-trophic interactions into analyses of food web stability and temporal dynamics may be crucial for their empirical reliability and predictive value.

Due to anthropogenic disturbances many important habitat modifier-dominated systems such as seagrass meadows, rainforests and coral reefs, are currently degrading worldwide at accelerating rates (e.g. Hodgson and Liebeler 2002; Foley *et al.* 2007; Waycott *et al.* 2009). Our study illustrates that the development of such diverse and complex communities can take decades due to the long-term accumulative effects of habitat modification. By showing that multiple species can affect food web dynamics across long timescales by interactively modifying the abiotic conditions, we illustrate that conservation and restoration efforts should not only focus on single species or interaction types. Instead, multiple species should be considered within an integrated network of multiple interaction types in order to adequately determine ecosystem responses to changing conditions.

Supplementary materials

Materials and Methods

Literature survey

To investigate the worldwide importance of habitat-modifying species in structuring food webs, we searched for examples of habitat modification on species distribution across multiple ecosystem types (table S1). Criteria for including a study were: (1) species richness was measured in the presence and absence of habitat modifying species, and (2) habitat modification affected different trophic levels (i.e. either different groups of species or different trophic levels within a group of species. The increase of species richness due to the presence of habitat-modifying species was calculated (%).

Remote sensing and GIS analyses

We used the normalized differences vegetation index (NDVI) obtained from Landsat 5 and 7 images (U.S. Geological Survey, resolution 30 m) to determine if and when seagrass colonized intertidal areas at the Banc d' Arguin. Images were available for the years 1973, 1985, 1994, 1999 to 2003, 2007, 2009 and 2010. Based on these analyses and after ground truthing in the field in 2011, we selected 4 replicates for three types of habitat: (1) bare habitat that was continuously bare from 1973 onwards, (2) colonizing seagrass habitat that was bare until 2007–2009 and was subsequently colonized by seagrass and (3) long-term stable seagrass habitat that had been continuously covered with seagrass from 1973 (table S2). All sites were selected based on a random spatial distribution (fig. S1) and similar environmental characteristics (see below).

To determine the change in intertidal pool size in long-term stable seagrass meadows, we analysed Google Earth images based on Quickbird (October 2004) and Geoeye (March 2011). Using Google Earth Pro, we measured the surface of 60 clearly visible pools at 5 long-term stable areas on the October 2004 image and subsequently, we measured the surface of the same pools again on the March 2011 image. All measured pools had comparable sizes to those observed in the long-term stable meadows during ground truthing (pool surface area < 75 m^2). The number of pools per ha and their relative cover was determined at each long-term stable site during field surveys.

Environmental characteristics

In order to exclude possible abiotic environmental effects in our study system, we tested whether environmental characteristics (e.g. inundation time, hydrodynamic conditions) differed between habitat types. We measured tidal elevation and distance to the gulley and determined an exposure index as a measure for hydro-

Table S1 Meta-analysis of studies investigating effects of habitat modifiers on species richness across multiple trophic levels.

Habitat	HM species	Focal community	Species richness +HM	Species richness -HM	Rel. effect	References
Marine						
Kelp forest	Giant kelp	Subtidal	274	176	1.57	Graham 2004
Intertidal mudflats	Blue mussels	Macrofauna and flora	25.6	3	8.53	Norling & Kautsky 2007
Intertidal mudflats	Blue mussels	Macrofauna and flora	10.6	7.1	1.49	Unpublished data Van der Zee *et al.* 2012
Intertidal mudflats	Sand mason worms	Macrofauna	37	22	1.68	Callaway 2006
Rocky shores	Purple mussels	Invertebrates	28.9	2.7	10.7	Silliman *et al.* 2011
Coral reefs	Coral	Fish	17.2	3.4	5.06	Cabaitan *et al.* 2008
Coral reefs	Coral	Fish	~12	~8	1.5	Syms & Jones 2000
Temperate seagrass meadows	Dwarf Eelgrass	Macrofauna	~24	~9	2.73	Do *et al.* 2011
Temperate seagrass meadows	Eelgrass	Macrofauna	30	11	2.67	Böstrom & Bonsdorff 1997
Tropical seagrass meadows	Tasman Grasswrack	Macrofauna	33	15	2.20	Edgar *et al.* 1994
Tropical seagrass meadows	Dwarf eelgrass & marbled swim crabs	Macrofauna	33.3	8.8	3.78	*
Cobble beaches	Smooth cordgrass & ribbed mussels	Macrofauna and flora	~5.5	~2.5	2.20	Altieri *et al.* 2007
Fresh water						
Forest headwater streams	Eastern hemlock	Aquatic invertebrates	47	32	1.47	Snyder *et al.* 2002
Dune slack ponds	Watermilfoil	Macrofauna	16.8	12.3	1.37	**
Terrestrial						
Oak/cypress forest	Spanish moss	Invertebrates	5.9	0.7	8.43	Angelini and Silliman 2014
Tropical forest	Leguminous trees & epiphytes	Insects	113	83.9	1.35	Cruz-Angón *et al.* 2009
Alpine zone	Cushion plants	Plant and arthropods	~7.3	~5.3	1.38	Molenda *et al.* 2012
Cottonwoods	Pale-headed Aspen leafroller moth	Arthropods	~ 11	~2	5.5	Martinsen *et al.* 2000
Mean increase					3.53	

*: this paper; **: unpublished data from our group.

dynamic exposure. We calculated the maximum and modified effective fetch length to calculate the exposure index (Howes et al. 1999). This index varies from 0 (very protected) to 5 (exposed). Results are shown in table S3.

Sediment and benthos field surveys

Field surveys were carried out on the intertidal mudflats near Iwik in the Parc National du Banc d' Arguin (PNBA)(19°52'42" N, 16°18'50" W, fig. S1) in January 2011. At each sampling site, a circle with a 50-m diameter was established prior to sampling. Within this circle, 4 sediment and benthos cores were taken and the silt layer height was measured 4 times with a gauge rod. Sediment cores were taken

Table S2 Habitat development over time of each site based on NDVI landsat 5 and 7 images (U.S. Geological Survey, resolution 30 m) for 1973-2010 and field observations in 2011 (B=bare sediment, S=vegetated with seagrass).

	Sites											
	Bare:				Colonizing:				Long-term stable:			
Year	B1	B2	B3	B4	C1	C2	C3	C4	L1	L2	L3	L4
1973	B	B	B	B	B	B	B	B	S	S	S	S
1985	B	B	B	B	B	B	B	B	S	S	S	S
1994	B	B	B	B	B	B	B	B	S	S	S	S
1999	B	B	B	B	B	B	B	B	S	S	S	S
2000	B	B	B	B	B	B	B	B	S	S	S	S
2001	B	B	B	B	B	B	B	B	S	S	S	S
2002	B	B	B	B	B	B	B	B	S	S	S	S
2007	B	B	B	B	S	B	B	S	S	S	S	S
2009	B	B	B	B	S	S	S	S	S	S	S	S
2010	B	B	B	B	S	S	S	S	S	S	S	S
2011	B	B	B	B	S	S	S	S	S	S	S	S

Table S3 Environmental characteristics. A one-way ANOVA was used for tidal elevation, distance to gulley, maximum fetch and modified fetch. A Kruskal Wallis test was used for exposure index.

	Mean values ± SE			Statistics		
	Bare (B)	Colonizing (C)	Long-term stable (L)	df (err.)	F or χ^2	*P*
Tidal elevation (NSI in cm)	-263±11	-247±15	-255±5	2 (9)	0.53	0.609
Distance to gulley (km)	0.09±0.03	0.14±0.04	0.90±0.03	2 (9)	0.66	0.541
Fetchmax (km)	7.77±2.02	8.61±1.20	20.85±10.45	2 (9)	1.69	0.238
Fetchmod (km)	0.76±0.24	0.82±0.18	4.97±3.12	2 (9)	3.35	0.082
Exposure index (class 0-5)	0.5±0.5	0.5±0.5	1.75±0.63	2	3.04	0.219

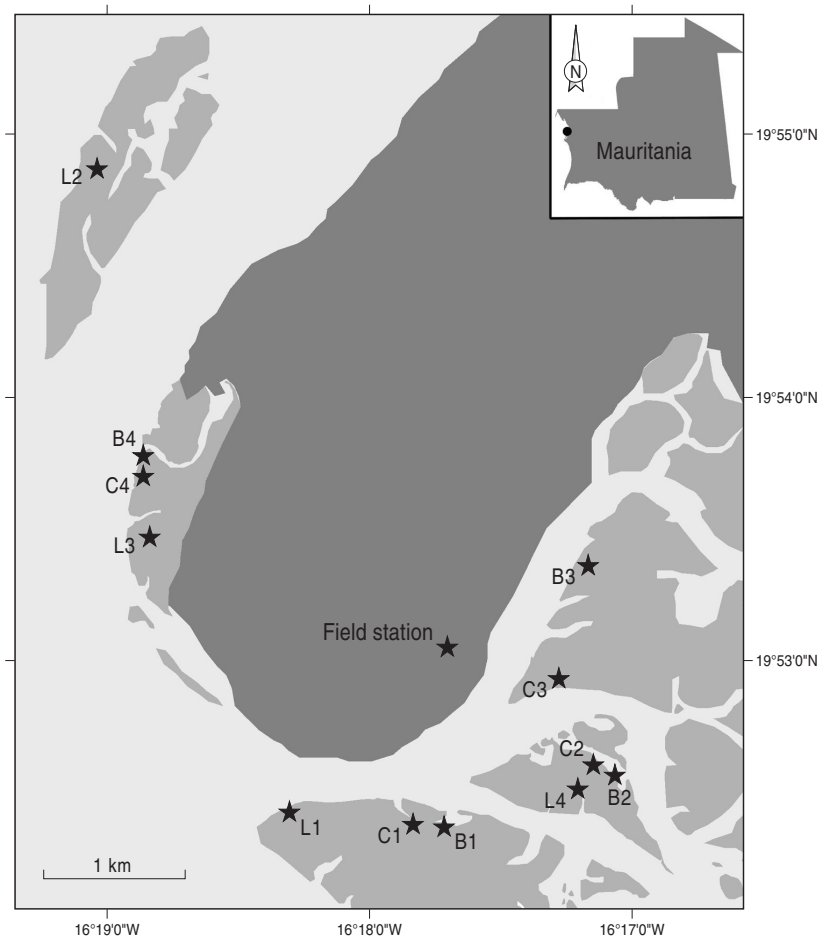

Figure S1 Map of the study area in the Banc d'Arguin, Mauritania. Light grey areas indicate water, intermediate grey areas indicate mudflats exposed during low water and land is represented by dark grey. Black stars represent the positions of the different study sites and the PNBA field station.

with a 12.5-cm^2 PVC corer to a depth of 5 cm. Organic matter content in freeze-dried sediment samples was estimated as weight Loss On Ignition (LOI; 5 h, 550°C). Silt content (% sediment fraction <63μm) was determined by a Particle size Analyzer (Malvern). Benthos samples were taken with a 179-cm^2 stainless steel core to a depth of 20 cm, after which the samples were sieved over a 1 mm mesh. To determine crustacean densities, four 5-m long hauls were taken with a shrimp net at each site. At the long-term stable sites, the water column of 4 intertidal pools was separately sampled. Crustaceans were sampled by taking 1 haul with a shrimp net from the edge to the centre of a pool, while fish were sampled by pulling a beach seine net through each pool. Additionally, depth and size was measured for each pool. All fauna was identified to species level in the laboratory.

Stable isotope measurements

Apart from the abovementioned species and sediment samples, we separately collected detritus and primary producers (algae, diatoms, seagrasses, epiphytes and detritus) in each area and obtained particulate organic matter samples from the water column (wPOM). wPOM was collected by filtering several litres of seawater over pre-combusted Whatman GF/F glass fibre filters, while sPOM (particulate organic matter from the sediment) was analysed from the sediment samples that were cleared of all living biomass. Benthic diatoms were scraped from the sediment surface. After migration through a mesh (80 Mu) into combusted sand, they were collected in filtered seawater and also filtered over glass fibre filters (Eaton and Moss 1966). We took muscle tissue samples from fish and soft tissue from invertebrates wherever possible, but used the whole animal for smaller samples. All material was rinsed with demineralised water, oven-dried at 50°C for 48 h and ground. We took sub-samples for separate carbon and nitrogen analyses when samples contained inorganic calcified structures. These samples were decalcified for carbon analysis by adding HCl. In total 10 fish species, 46 invertebrate species, 6 primary producers, sPOM, wPOM and zooplankton were analysed for isotopic signature.

Burrowing crabs: abundance and behaviour

At each site, two transects (50 m long and 10 wide) were established and marked with pvc- poles at the corners. The number of crab (*Callinectes marginatus*) holes within these transects was used as a proxy for crab density and activity per site on the emerged tidal flats. At three of our long-term stable sites (L1, L3 and L4), we measured pool size and depth of 18, 31 and 11 intertidal pools, respectively. Within these pools, the number of crab holes was scored to determine the average crab density per pool and the relation between number of crabs per pool and pool size (fig. S2). Both measurements were combined to determine the total amount of crab holes per habitat type (fig. 7.1E & table S4).

In an additional cage-experiment, we determined whether crabs were able to dig holes in the silt layer of a healthy, undisturbed seagrass meadow. A circular cage (50 cm in height with a diameter of 125 cm and mesh size of 1 cm; fig. S3) was pushed into the sediment with full seagrass cover, to a depth of approximately 25 cm. Crabs with an average carapace width of 7.6 ± 0.4 cm were caught in the direct surroundings and released in separate cages (n = 3). The length and width of present crab holes were measured after 24 hours.

Fish abundance

Ray abundance on the tidal flats was determined through visual surveys from a boat along random transects, within 10 meters of each side of the front of the boat at a maximum speed of 5 km h^{-1}. Transect lengths were determined using GPS. Fourteen transects (~250–300 m long) were carried out by boat at sites L1, L3 and L4 (42

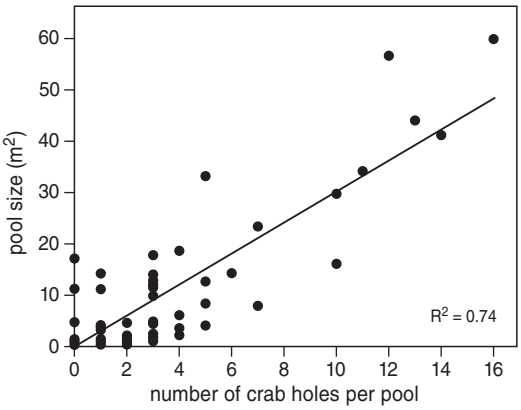

Figure S2 Positive linear relationship (y = 3.04x, t = 17.1, $R^2 = 0.74$, $P < 0.001$) between number of crabs per pool and pool size (m^2).

transects in total) in October 2012. Rays were identified up to species level whenever possible, but other taxonomic classes (family, order) were occasionally used when identification up to species level was not possible. We used the average number of rays per transect per site as a measure of abundance per hectare on the tidal flats (table S5).

Table S4 Habitat characteristics with ANOVA values and Tukey-Posthoc groups (B=bare, C=colonizing, L=long-term stable habitat) for seagrass cover, organic matter, grain size and depth of the silt layer. To obtain normality of variance of residuals, organic matter was reciprocally transformed (y = 1/x) and grain size and depth of the silt layer were both log transformed (y = log10(x)). Shoot density and crab density were not normally distributed, also not after transformation and for these habitat characteristics a Kruskal Wallis test was used in combination with Mann-Whitney U tests. Sediment organic matter, grain size and the silt layer were measured in seagrass patches and in bare patches, but for statistical analysis bare patches and seagrass patches were averaged. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Mean values ± SE			Statistics			
	Bare (B)	Colonizing (C) bare/seagrass patches	Long-term stable (L)	df (err.)	F or χ^2	*P*	Posthoc groups (B, C, L)
Seagrass cover (%)	0±0	46.3±10.3	87.5±3.2	2 (9)	49.2	***	a, b, c
Shoot density (# m^{-2})	0±0	6246±510	7167±575	2	8.3	*	a, b, b
Organic matter (%)	1.7±0.2	1.6±0.2/2.1±0.2	8.6±0.4	2 (9)	47.4	***	a, a, b
Grain size (% <63μm)	13.0±2.6	11.6±1.2/16.6±3.1	71.3±4.2	2 (9)	40.4	***	a, a, b
Depth silt layer (m)	0±0	0.05±0.0/0.1±0.0	0.9±0.2	2 (9)	98.3	***	a, b, c
Crab density (# ha^{-1})	0±0	308±54	3300±427	2	9.3	**	a, b, c

Figure S3 A circular crab enclosure (50 cm in height (25 cm belowground) with a diameter of 125 cm and mesh size of 1 cm) was used to test whether crabs were able to create holes in a healthy undisturbed seagrass meadow. (Photo: Marjolijn Christianen).

To determine the abundance of rays and other potentially bioturbating fish species in the intertidal pools, we placed underwater cameras (GoPro HD HERO2, Woodman Labs Inc, USA) in pools at sites L1, L3 and L4. Per site, the size of three pools were measured and subsequently monitored by cameras taking photographs at 10-s intervals across one full high tide period. All fish species that entered the pools were marked as potentially bioturbating species. Each fish was identified to species level whenever possible and other taxonomic classes (family, order) were used when identification up to species level was not possible. Average numbers per pool for each species were used to calculate the abundance per hectare per site in the pools (table S5).

Shorebird abundance

Shorebird abundance was measured in a 60×100 m square (0.6 ha, marked with PVC poles) at sites L1, L3 and L4. During low tide, the number of feeding shore birds was scored every 15 minutes in the square from a distance of ~400 m, using a telescope (zoom ocular 20–60×; ATM 80 HD, Swarovski, Absam, Austria). Counting started when the water had retreated from the marked square until the area was inundated again. Birds were counted during three complete tidal cycles in October 2012. We used the average number of birds as a measure of abundance per hectare on the tidal

flats (table S6). When shorebirds were encountered in our marked square, we scored whether they were foraging in or outside the intertidal pools. Average numbers per pool for each species were used to calculate the abundance per hectare in the pools (table S6).

Food web analyses

Based on abundance and size data, literature, the WoRMS (World Register of Marine Species) database and connected online databases, we determined potential trophic relations for each species and constructed a maximized interaction matrix for each site that included all potential trophic links. Next, we used stable isotope biplots and Bayesian mixing models (R-package SIAR) per site for all consumers to estimate of the relative contribution of potential resources to the consumer's diet. Based on these analyses, we removed trophic links where a resource contributed less then 5%

Table S5 Ray and other fish densities (number ha^{-1}) at three long-term stable sites. Densities were determined for the number of rays on the tidal flats and for the number of rays and other fish species present in the pools at the same tidal flat.

	Mean values ± SE			
Ray-Fish species	**Site L1**	**Site L3**	**Site L4**	**Total**
TIDAL FLAT				
Dasyatis marmorata	0.11±0.11	0±0	0.85±0.85	0.32±0.27
Rhinobatos sp	1.09±0.43	0.79±0.37	0.78±0.53	0.89±0.10
Total				0.60±0.28
POOL				
Dasyatis marmorata	0.23± 0.15	0.43±0.43	0.18±0.18	0.28±0.08
Rhinobatos sp	0.31±0.25	0.04±0.04	0.09±0.06	0.15±0.08
Total				0.21±0.07
Arius heudelotii	0.19±0.11	2.88±2.77	7.31±3.99	2.28±1.07
Dicentrarchus punctatus	1.34±1.34	2.88±1.95	0.15±0.15	1.46±0.79
Diplodus sp	4.80±3.80	6.55±6.55	0.03±0.03	3.80±2.00
Ephippion guttiferum	0.27±0.12	0.60±0.60	0.02±0.02	0.30±0.17
Epinephelus aeneus	0.31±0.26	0.94±0.94	7.05±3.55	2.76±2.5
Epinephelus sp	0±0	0±0	1.18±0.54	0.39±0.39
Mugil cephalus	3.51±3.45	33.36±32.38	26.97±19.87	21.28±9.07
Rhyzoprionodon acutus	0.04±0.04	0±0	0±0	0.01±0.01
Sciaena umbra	0.04±0.04	0.04±0.04	1.13±0.88	0.40±0.36
Tilapia guineesis	0±0	28.60±28.40	0±0	9.54±9.54
Umbrina canariensis	0±0	0±0	7.90±6.60	2.63±2.63
Total				4.08±1.90

to the diet of the consumer. Finally, we used the obtained interaction matrix to calculate species number (S), number of links per species (L/S) and connectance (C; defined as L/S^2).

To elucidate the effects of hierarchical habitat modification by seagrass and burrowing crabs on the food web structure we excluded modification by crabs and seagrass in a stepwise manner. Based on the physiological constraints of each species, we first constructed a modification-dependence matrix in which the dependence of each species on modification by seagrass (aboveground structure, silty sediment) and crabs (intertidal pools) was expressed as either 1 (dependent) or zero (not dependent). Based on these data, we then removed all species dependent on intertidal pools from each stable site and reconstructed the trophic-interaction matrix. Next, we re-calculated species-richness, the number of links per speciesa and connectance, based on the reconstructed interaction matrix. Finally, this process was repeated for species dependent on modification by seagrasses.

Table S6 Shorebirds densities (number ha^{-1}) at three long-term stable sites. Densities were determined for the number of shorebirds foraging on the tidal flats and for the number of birds foraging in the pools within the same tidal flat.

	Mean values ± SE			
Shorebird species	**Site L1**	**Site L3**	**Site L4**	**Total**
TIDAL FLAT				
Phoenicopterus roseus	0±0	0±0	0.04±0.04	0.01±0.01
Pelecanus onocrotalus	0.04±0.04	0±0	0±0	0.01±0.01
Egretta gularis	0.59±0.21	0.16±0.16	0.48±0.14	0.36±0.18
Ardea cinerea monicae	0.07±0.04	0±0	0.12±0.06	0.06±0.03
Egretta garzetta	0.09±0.09	0.09±0.06	0.04±0.03	0.07±0.01
Platalea leucorodia (*balsaci*)	0±0	0.03±0.02	0.14±0.09	0.06±0.04
Numenius arquata	0.69±0.11	0.44±0.19	1.27±0.40	0.80±0.24
Limosa lapponica	2.71±0.66	1.09±0.37	7.07±2.67	3.62±1.79
Total				0.63±0.22
POOL				
Phoenicopterus roseus	0±0	0±0	0.04±0.04	0.01±0.01
Pelecanus onocrotalus	0±0	0±0	0±0	0±0
Egretta gularis	0.09±0.06	0±0	0.11±0.04	0.07±0.03
Ardea cinerea monicae	0±0	0±0	0±0	0±0
Egretta garzetta	0±0	0.05±0.03	0±0	0.02±0.02
Platalea leucorodia (*balsaci*)	0±0	0.03±0.02	0.07±0.07	0.03±0.02
Numenius arquata	0.22±0.09	0.13±0.07	0.16±0.09	0.17±0.03
Limosa lapponica	0.18±0.05	0.13±0.05	0.57±0.49	0.29±0.14
Total				0.07±0.02

To elucidate the trophic effects of burrowing crabs and seagrass on the food web structure, we repeated the procedure described above by excluding species from the matrices that were trophically dependent on crabs and seagrass in the same stepwise manner. An overview of the results is shown in table S8.

Statistical analyses

Normality of variance of residuals was checked with the Shapiro-Wilks test ($P = 0.05$) and further confirmed by graphical validation of the final models. If the normality assumption was not met, data were transformed or non-parametric tests were used. All relevant transformations are mentioned in the figures or table legends in the supplementary materials. The difference in pool size over time was analyzed with a paired samples t-test. Environmental and habitat characteristics were analyzed with a one-way ANOVA, followed by a post-hoc Tukey HSD test, or with a Kruskal Wallis test, followed by Mann-Whitney U tests. To correct for the multiple testing problem during the Mann-Whitney U tests, significance levels were adjusted based on the false discovery rate control (Benjamini and Hochberg 1995). An overview of the statistical output from these analyses is provided in tables S3 and S4. The food web analyses were done with a one-way ANOVA, followed by a post-hoc Tukey HSD test (see table S8).

Supplementary text

To investigate whether other animals play a significant role in the formation and maintenance of the intertidal pools, we determined densities of possible bioturbating fish and shore birds species (see Materials and Methods). Based on literature, we suspected rays to be one of the more important bioturbating species (Orth 1975; Townsend and Fonseca 1998). We found an average density of 0.60 ± 0.28 rays ha^{-1} on the intertidal flats and an average density of 0.21 ± 0.07 rays ha^{-1} in the pools (table S5). In addition, observations with underwater cameras in the pools yielded an average of 4.08 ± 1.90 individuals ha^{-1} pool of other potentially bioturbating fish (table S5). Apart from fish, we also investigated the presence of shorebirds as possible bioturbating species. Only bird species that were large enough to walk in the pools or could enter the pools by swimming or diving were counted during our observations (table S6). We found an average density of 0.63 ± 0.22 birds ha^{-1} on the tidal flats and an average density of 0.07 ± 0.02 birds ha^{-1} in the tidal pools (table S6). Overall, these results suggest that both fish and bird numbers were too low to explain the high numbers of intertidal pools (82.8 ± 9.2 pools ha^{-1}), especially in comparison to the high number of burrowing crabs (3151 ± 500 ha^{-1}) present in the area and their strong correlation with pool size (fig. S2).

Table S7 Feeding modes.

Species	Group	Feeding mode	Reference
Algae	Algae	-	Round 1984
Abra tenuis	Bivalve	deposit feeder, sPOM, diatoms, detritus/debris	Hughes 1973
Anadara senilis	Bivalve	suspension feeder	Yoloye 1975
Diplodonta diaphana	Bivalve	suspension feeder, wPOM, plankton, sPOM	Macdonald *et al.* 2010, Arruda *et al.* 2003
Dosinia isocardia	Bivalve	suspension feeder, wPOM, plankton, sPOM	Rodhouse and Roden 1987
Loripes lacteus	Bivalve	symbiose met bacterien, suspension feeder	Johnstone *et al.* 1994
Petricola sp	Gastropods	suspension feeder	Appeltans *et al.* 2009
Tellina sp	Bivalve	deposit feeder, sPOM, diatoms, detritus/debris	Yonge 1949, Compton *et al.*2008
Ampithoe sp	Crustacea	seagrass, detritus, epiphytes, algae	Mukai and Iijima 1995, Robertson and Mann 1980
Callinectes marginatus	Crustacea	omnivore: eats everything from plant material, detritus to fish	Laughlin 1982, Mascaro *et al.* 2007
Carcinus mediterraneus	Crustacea	amphipods, isopods, polychaetes, gastropods, bivalves, seagrass, alge, detritus	Baeta et al 2006, Chen *et al.* 2004
Idotea chelipes	Crustacea	omnivore: algae, animal tissue, other isopods, epiphyton	Naylor 1955, Hootsmans and Vermaat 1985
Palaemon sp	Crustacea	amphipods, isopods, polychaetes, gastropods, bivalves, seagrass, sPOM,	Guerao and Ribera 1996, Berglund 1980
Diatoms	Diatoms	-	Barranguet *et al.* 1997
Epiphyton	Epiphytes	nutrients also from seagrass leaves	Orth and Van Montfrans 1984 and references therein
Conger conger	Fish	osteichthyes, crustacean, cephalopoda, polychaeta	Cau and Manconie 1984, O'Sullivan *et al.* 2004
Atherina presbyter	Fish	small crustacea and fish larvae	Quignard and Pras 1986
Diplodus sargus	Fish	omnivore: algae, sea urchins, polychaetes, gastropods and amphipods	Figueiredo *et al.* 2005
Eucinostomus melanopterus	Fish	amphipods, isopods, crabs, bivalves, gastropods, juvenile fish, detritus, diatoms, algae	Gning *et al.* 2010
Mugil cephalus	Fish	algae, diatoms, crustaceans, polychaetes, bivalves, fish, detritus, zooplankton	Soyinka 2008, Blay 1995, Michaelis 1993
Pomatoschistus microps	Fish	polychaetes, bivalves, amphipods, isopods, shrimps, juvenile fish	Salgado *et al.* 2004, Leitao *et al.* 2005
Solea senegalensis	Fish	polychaetes, bivalves, gastropoda, amphipods, isopods	Kostecki *et al.* 2012, Teixeira and Cabral 2010
Stephanolepis hispidus	Fish	amphipods, bivalves, isopods, decapods, gastropods,algae, detritus, seagrass leaves	Prado and Heck 2011
Syngnathus typhle	Fish	amphipods, isopods, shrimps, polychaetes, bivalves, gastropoda, juvenile fish, algae	Oliveira *et al.* 2007
Tilapia guineensis	Fish	sPOM, juvenile fish, zooplankton, aquatic invertebrates, detritus, bivalves, shrimp	Louca *et al.* 2010
Bittium reticulatum	Gastropods	microalgal grazers, periphyton	Rueda *et al.* 2009
Bulla adansoni	Gastropods	algae, diatoms, seagrass, copepods, foraminiferans, larvae of molluscs, epiphytes	Malaquias *et al.* 2008
Clavatula bimarginata	Gastropods	omnivore	WoRMS 2009
Columbella rustica	Gastropods	herbivore: algae, diatoms, epiphytes, live on seagrass	deMaintenon 1999
Crepidula goreensis	Gastropods	filter feeder: diatom particles, wPOM and sPOM	Rueda *et al.* 2009, Orton 1912
Dendrodoris sp	Gastropods	sponges, epiphyton	Zhukova and Eliseikina 2012

Species	Group	Feeding mode	Reference
Elysia sp	Gastropods	herbivore that feeds on seagrass and algae	Clark 1975, Jensen 1983
Gibbula umbilicalis	Gastropods	herbivores that feeds on epiphytes and algae	Steneck and Watling 1982 and references therein
Nassarius miga	Gastropods	omnivore, scavenger, surface deposit feeder	Morton and Jones 2003
Smaragdia viridis	Gastropods	seagrass	Holzer *et al.* 2011, Rueda *et al.* 2009
Tricola pulla	Gastropods	diatoms, detritus, algae, microalgal or periphyton grazers	Rueda *et al.* 2009
Oligochaeta	Oligochaete	deposit feeders, sPOM, detritus, diatoms	Giere 2006
Aphelocheata sp	Polychaeta	surface deposit feeder, transporting sand, debris and diatoms	Farke 1979
Euclymene sp	Polychaeta	subsurface deposit feeder, detritus, sPOM	Chardy and Dauvin 1992, Martin *et al.* 2000
Euphrosine sp	Polychaeta	carnivore: sponges, foraminiferans	Fauchald and Jumars 1979 and references therein
Glycera alba	Polychaeta	carnivore on other worms	Fauchald and Jumars 1979, Ockelmann & Vahl 1970
Harmothoe sp	Polychaeta	carnivore, small crustaceans(amphipods), gastropods, polychaetes	Fauchald and Jumars 1979, Sarvala 1971
Heteromastus filiformis	Polychaeta	surface deposit feeder, transporting sand, debris and diatoms, algae, detritus	Fauchald and Jumars 1979, Macdonald *et al.* 2010
Lanice sp	Polychaeta	detrivore, detritus, diatoms, sPOM, suspension feeder, wPOM	Fauchald and Jumars 1979, Buhr 1976
Marphysa sanguinea	Polychaeta	omnivore	Fauchald and Jumars 1979
Nephtys sp	Polychaeta	omnivore: molluscs, crustaceans and other polychaetes, diatoms, detritus, sPOM	Fauchald and Jumars 1979, Arndt and Schiedel 1997
Nereis sp	Polychaeta	predator and filter feeder: sPOM, wPOM, algae, diatoms, other worms, small isopods	Fauchald and Jumars 1979 and references therein
Ophelia sp	Polychaeta	deposit feeders, sPOM, detritus, diatoms	Fauchald and Jumars 1979 and references therein
Petaloproctus terricola	Polychaeta	detritus, diatoms, small animals, suspension feeding, wPOM, sPOM, zooplankton	Fauchald and Jumars 1979 and references therein
Polydora antennata	Polychaeta	deposit feeders, sPOM, detritus, diatoms, suspension feeder wPOM, zooplannkton	Fauchald and Jumars 1979, Macdonald *et al.* 2010
Scolelepis squamata	Polychaeta	deposit feeder, suspension feeder, wPOM, sPOM, diatoms, zooplankton	Fauchald and Jumars 1979, Dauer 1983
Scoloplos capensis	Polychaeta	deposit feeder	Fauchald and Jumars 1979, Rice *et al.* 1986
Terebellidae sp	Polychaeta	deposit feeder, sPOM, diatoms, detritus/debris	Fauchald and Jumars 1979 and references therein
Cymodocea nodosa	Seagrass	-	Hemminga *et al.* 1991 and references therein
Halodule wrightii	Seagrass	-	Hemminga *et al.* 1991 and references therein
Zostera noltii	Seagrass	-	Hemminga *et al.* 1991 and references therein
Zooplankton	zooplankton	wPOM, sPOM, zooplankton	Suthers and Rissik 2009

Table S8 Change in food web structure indicators due to stepwise removal of (1) non-trophic facilitation effects (i.e., habitat modification) and (2) trophic effects (i.e., direct removal of the species from the network) of of crabs and seagrass respectively. Table shows ANOVA values and Tukey-Posthoc groups (B=bare, C=colonizing, L=long-term stable habitat). Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Mean values ± SE			Statistics		
	Long-term stable (L)	Colonizing (C)	Bare (B)	F	*P*	Posthoc groups (L, C, B)
All included						
Number of species (S)	35.25±1.65	15.50±0.65	10.25±0.85	134.5	***	a, b, c
Links per species (L/S)	4.71±0.11	3.37±0.12	2.25±0.17	46.35	***	a, b, c
Connectance (L/S^2)	0.13±0	0.22±0.01	0.22±0.01	74.18	***	a, b, b
- Crab facilitation						
Number of species (S)	14.50±0.50	15.50±0.65	10.25±0.85	16.70	***	a, a, b
Links per species (L/S)	2.92±0.05	3.37±0.12	2.25±0.17	20.23	***	a, a, b
Connectance (L/S^2)	0.20±0.01	0.22±0.01	0.22±0.01	2.06	ns	a, a, a
- Seagrass facilitation						
Number of species (S)	11.00±0.58	12.50±0.5	10.25±0.85	3.00	ns	a, a, a
Links per species (L/S)	2.18±0.09	2.46±0.09	2.25±0.17	1.16	ns	a, a, a
Connectance (L/S^2)	0.20±0.01	0.20±0.01	0.22±0.01	1.29	ns	a, a ,a
- Crabs						
Number of species (S)	34.25±1.65	14.50±0.65	10.25±0.85	127.0	***	a, b, b
Links per species (L/S)	4.27±0.23	2.96±0.10	2.25±0.17	35.33	***	a, b, c
Connectance (L/S^2)	0.13±0	0.21±0.01	0.22±0.01	117.4	***	a, b, b
- Seagrass						
Number of species (S)	33.25±1.65	13.50±0.65	10.25±0.85	120.0	***	a, b, b
Links per species (L/S)	4.21±0	3.10±0.09	2.25±0.17	30.88	***	a, b, c
Connectance (L/S^2)	0.13±0	0.23±0.01	0.22±0.01	105.9	***	a, b, b